

NEWS FROM AMRIS

The Advanced Magnetic Resonance Imaging and Spectroscopy Facility at the University of Florida

Microcoil NMR Technology for Small Samples of Proteins

Y. Li, AMRIS/UF, Biochemistry and Molecular Biology

X. Zhang, Univ. of Illinois at Urbana-Champaign, Electrical and Computer Engineering

T. Logan, NHMFL/FSU, Chemistry and Biochemistry

A. Webb, Univ. of Illinois at Urbana-Champaign, Electrical and Computer Engineering

A.S. Edison, AMRIS/UF, Biochemistry and Molecular Biology

NMR suffers from poor sensitivity, because the frequencies required for resonance are low and the ground and excited states are nearly equally populated at room temperature. This can be improved by increasing the frequency (e.g., making higher field magnets), lowering the temperature (e.g., high B/T), or improving the measurement efficiencies. The NHMFL has great strengths in all three areas, and this report summarizes recent developments in improvements in measurement efficiencies through the design of small radio frequency (RF) solenoidal microcoils.

The signal to noise (S/N) in an RF coil is approximately inversely proportional to the diameter of the coil. Thus, by decreasing the coil diameter, the S/N per nuclear spin increases. Of course, this increase in S/N is at a cost of sample volume. The optimal type of sample for microcoils is one that is mass-limited (e.g., hard to get) but soluble in relatively high concentrations. Microcoils have been widely used for small molecule, natural product NMR due to the difficulty in obtaining large quantities of material. Protein NMR has traditionally used larger 5 mm samples, because many proteins tend to aggregate at high concentrations. However, the high costs of fully labeling proteins with ^{13}C , ^{15}N , and sometimes ^2H , along with the difficulty often encountered in producing them make them, ideal candidates for microcoil NMR.

We started our work on protein solenoidal microcoils a few years ago with a relatively large coil of 2.5 mm and an active sample volume of about 15 μL (Li, *et al.*, 2003). This single coil was tuned to 4 frequencies, ^1H , ^{13}C , ^{15}N , and ^2H , allowing for standard protein triple resonance experiments to be collected. One significant benefit from the single microcoils tuned to multiple frequencies is that 90 degree pulse lengths are extremely short for all channels. Fig. 1 shows a comparison of two ^{15}N -HSQC spectra collected with the same total amount of material with 10x more concentration but 10x less volume in the microcoil. The S/N benefit in going smaller is apparent and suggests that this technology is appropriate for proteins.

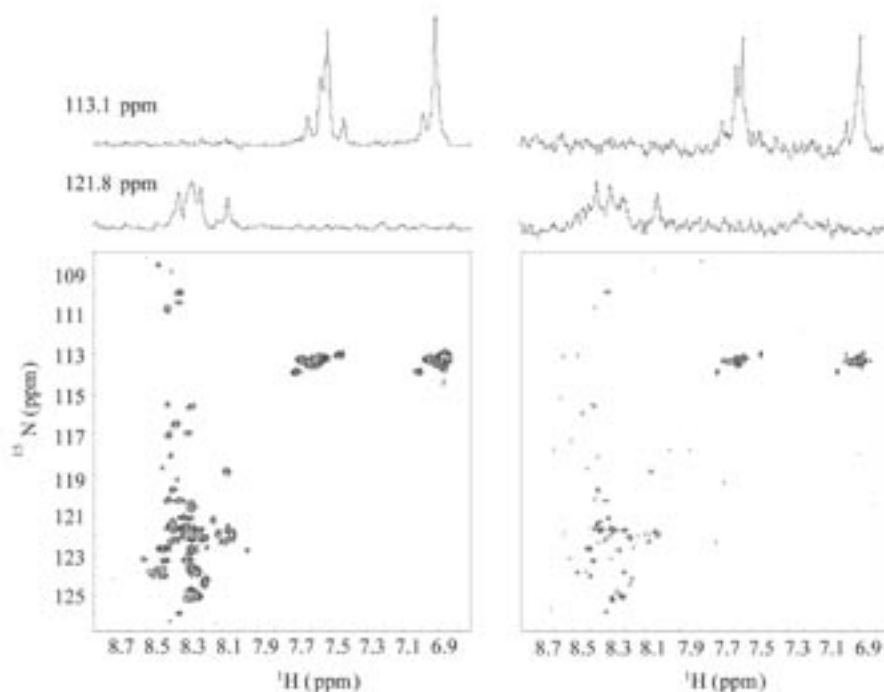


Figure 1. Comparison of the 2.5 mm solenoidal probe and a commercial 5 mm probe using equal amounts of ^{15}N -labeled IA-3: the data were acquired using a $^1\text{H}\{-^{15}\text{N}\}$ HSQC sequence. The solenoidal probe contained 60 μL of 1 mM ^{15}N -labeled IA-3, and the 5 mm probe contained 600 μL at a concentration of 0.1 mM. Two 1-D slices at 113.1 and 121.8 ppm in the ^{15}N dimension are shown on top of the 2D spectrum: spectra from the 5 mm probe are shown at a 10-times higher scale. The signal-to-noise ratios of the maximum peaks in these slices are 130:1 and 13:1 (113.1 ppm) and 60:1 and 6.5:1 (121.8 ppm). Complete details can be found in Li *et al.*, 2003.

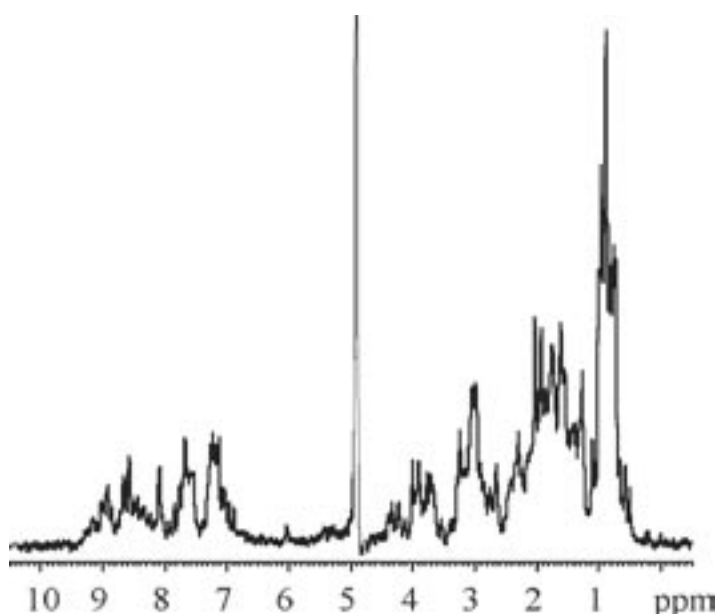


Figure 2. 1D ^1H NMR spectrum of 1 μL active volume of 3 mM Target 2 protein. The spectrum was obtained at 750 MHz with 8 scans and used Watergate for water elimination.

Given our initial encouraging results from a 2.5 mm coil, we have recently decided to further push the limits with a 1 mm solenoid microcoil probe that has an active sample volume of about 1 μL and a total volume of about 4 μL . Both simulation and experimental methods have been utilized to optimize this coil. In order to improve the homogeneity of radio frequency field, a large length to diameter ratio of ~ 2.3 was used in the 1 mm solenoid microcoil design. We also found that the use of rectangular wires instead of round wires in the winding of such a coil can considerably reduce the coil loss and improve the sensitivity performance in NMR experiments. A 1D ^1H NMR 750 MHz spectrum of a protein demonstrating the high S/N using this coil is shown in Fig. 2.

In collaboration with Jim Prestegard's laboratory at the University of Georgia, we have collected several 3D triple resonance data sets of "Target-2" protein using the optimized 1 mm solenoid coil. Our immediate goal is to sequentially assign the protein using just 4 μL of sample, and we also will collect data to determine a low-resolution structure of the protein. The amount of the protein sample for these studies is below 10 nano-moles. The 3D NMR spectra have been acquired on a 750 MHz Bruker spectrometer in the AMRIS facility.

A significant advantage of microcoil technology is the ability to collect several datasets simultaneously because of the small size of the coils (Li *et al.*, 1999). This technique has been recently applied to 8 small molecules using a ^1H probe (Wang *et al.*, 2004). Simultaneous protein NMR is more challenging than small molecule studies, because the coils need to be larger and there are several additional frequencies that are required for protein NMR that need to be electrically isolated. Fig. 3 demonstrates the concept with two protein ^{15}N -HSQC spectra simultaneously collected with two 2.5 mm solenoid coils in the same probehead. By expanding this technique to more samples, this technology can easily be

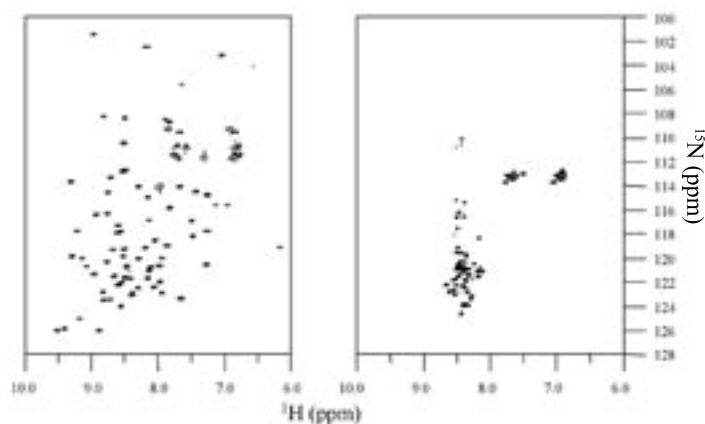


Figure 3. Simultaneous protein NMR detection using solenoids. (left) ^{15}N HSQC spectrum of 1.25 mM ^{15}N -labeled ubiquitin in 90% $\text{H}_2\text{O}/10\%$ D_2O , 50 mM phosphate buffer, pH 5.5. Data acquisition parameters: $\text{sw}=4000$ Hz, $\text{sw}_1=1600$ Hz, 1024 complex data points, 192 t_1 increments acquired in States mode, 1 s water presaturation, 32 signal averages. Total data acquisition time 3.5 hours. (right) ^{15}N HSQC spectrum of 1 mM ^{15}N -labeled IA-3 in 90% $\text{H}_2\text{O}/10\%$ D_2O , 50 mM phosphate buffer, pH 5.5. Identical data acquisition parameters were used. Data were acquired in interleaved fashion with pulse transmission and data reception routed through an RF switch controlled from the console.

applied to high-throughput structural genomics, screening of large numbers of proteins for folding, and molecular library screening of molecules binding to proteins in drug discovery. This study is especially relevant to the ultra-wide bore 900 MHz magnet that will have room for larger arrays of microcoils than any other high-field magnet available today.

In summary, microcoils can provide very high quality data for proteins that are comparable in resolution to data collected on standard 5 mm commercial probes but that have much higher S/N per unit spin. These probes have exceptionally short pulse widths on all channels, making them ideal for protein NMR, especially using ^{13}C , at high magnetic field strengths. Their size allows the development of novel approaches to multiple sample detection, which has important applications in structural genomics and drug development. Finally, microcoil probes are relatively inexpensive and easy to construct and can be designed and constructed for particular sample needs.

¹ Li, Y., *et al.*, *Anal. Chem.*, **71**, 4815-4820 (1999).

² Li, Y., *et al.*, "Design and Optimization of High Sensitivity Small Volume Probes for Improved Limits of Detection in Protein NMR experiments" *Journal of Magnetic Resonance* **164**, 128-135 (2003).

³ Wang, H., *et al.*, *In Press, Journal of Magnetic Resonance* (2004).

⁴ Li, Y., *et al.*, ENC, Monterey CA, April 19-23 (2004).

This work was supported by "High Field Magnetic Resonance Research and Technology" 05/01/01 - 04/30/06 NIH/NCRR P41 RR16105-01 (PI: Blackband; Core PIs: Edison, Blackband, and Fitzsimmons).